Influence of Temperature and Wetness Duration on Sporulation of *Phytophthora cactorum* on Infected Strawberry Fruit

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ABSTRACT

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Strawberry fruits (cultivar Tristar) infected with *P. cactorum* were used to determine the effects of temperature and wetness duration on sporangial production. Sporangial production increased with increased wetness duration (3–24 hr) between 12.5 and 27.5 C. For each wetness duration, sporangia production increased up to the optimum temperature (about 20 C) and then declined. Sporangia were present after 3 hr of wetness between 15 and 25 C; 100 or more sporangia were produced at these temperatures with ≤16 hr of wetness. A multiple regression model was developed to describe sporangial production as a function of temperature and wetness duration.

Additional key words: disease forecasting, *Fragaria × ananassa*, quantitative epidemiology.

Epidemics of leather rot of strawberry (*Fragaria × ananassa* Duch.), which are caused by *Phytophthora cactorum* (Leb. & Cohn) Schroet., have resulted in significant quality and yield losses in Ohio's fruit crop in recent years. Excessive rainfall and/or flooded conditions are associated with disease development (2,13).

Numerous sporangia characteristic of *P. cactorum* have been observed microscopically on the surfaces of infected fruit collected either during or immediately after wetness periods in the field (2,12). Dispersal of sporangia by water splash mechanisms has been demonstrated (4). These observations have indicated that under favorable environmental conditions, a potential source of secondary inoculum readily forms on infected fruit surfaces. Rainfall following such conditions could result in epidemics of the severity experienced in Ohio during recent years.

Our investigations have indicated that a film of water is necessary for sporangial production by *P. cactorum* on infected strawberry fruit (*unpublished*). Environmental conditions (i.e., wetness duration and temperature) conducive to infection, as well as a regression model that accurately predicts infection at different temperatures and wetness durations, have been described (5).

The purposes of this study were: to determine the temperatures and wetness durations necessary for sporangial production on the surface of infected fruit, and to develop a model to predict sporangial production at different temperatures and wetness durations.

MATERIALS AND METHODS

Inoculum production and host inoculation. Day-neutral strawberry plants (cultivar Tristar) were used in all experiments. Plants were grown to reproductive maturity in a mixture of peat, sand, and steam-disinfested silt-loam (1:1:1, v/v) in the greenhouse. For each temperature×wetness duration combination that was tested, 48 detached, immature (green) fruit were washed with five 500-ml aliquots of sterile distilled water, surface sterilized in 5% Clorox for 30 sec, rinsed five times with 500-ml aliquots of sterile distilled

water, and inoculated with a zoospore suspension of *P. cactorum*. All inoculations were performed with cultures freshly isolated from infected strawberry fruit (cultivar Tristar) on pentachloro-nitrobenzene-benomyl-neomycin sulfate-chloramphenicol (PBNC) medium (16). Mycelial transfers from the edges of 3-day-old cultures were made to lima bean broth (16). Broth cultures were incubated 96 hr at 22 C in continuous light at 2.7 W/m². Cultures were then refrigerated for 30 min at 5 C, then returned to 22 C. Zoospores were released approximately 30 min after removal from refrigeration. All inoculations were made with zoospore suspensions (10,000/ml) in sterile distilled water brought to final concentration by measurement with a hemacytometer. Fruits were placed in 30-ml of inoculum contained in 15-cm-diameter plastic petri plates and incubated at 22 C in continuous light at 2.7 W/m² for 4 hr. Inoculated fruit were then rinsed with five 500-ml aliquots of sterlied distilled water to remove any mycelia, zoospores, or sporangia adhering to the fruit surface and then air-dried for 1 hr at 22 C at 30–40% RH in continuous light at 2.7 W/m².

Incubation. After their surfaces had dried, the fruits were placed on elevated screens contained in 1-L clear-glass jars and incubated for 72 hr at 20 C in 70% ± 5% RH in a 14-hr photoperiod at 99 W/m². Relative humidity in the incubation jars was controlled with glycerol:water mixtures in a closed system (6,11,15). Preliminary experiments indicated that a constant RH during the incubation period reduced the variability in subsequent sporangial production. The entire apparatus (i.e., jars, tubing, air filter, and pump) was placed in a controlled-environment chamber (Environmental Growth Chambers, Chagrin Falls, OH) at 20 ± 0.5 C. Incubation jars were positioned horizontally to ensure uniform lighting. Temperature and relative humidity within the closed systems were continuously monitored with sensors (Phys-Chem Sensor; Phys-Chemical Research Corporation, New York, NY) connnected to a microprocessor-controlled datalogger (CR-21, Campbell Scientific, Logan, Utah). Temperature in the controlled-environment chamber was continously monitored with thermistors (Fenwall Electronics, Ashland, MA), also connected to the datalogger.

Sporangial production. After 72 hr of incubation, inoculated fruit were exposed to various wetness durations in a second controlled-environment chamber containing a 1 m³ clear-plastic chamber enclosing a Herrmidifier mist generator (Herrmidifier Co., Lancaster, PA 17604). This chamber will be called the mist

chamber. Each fruit was placed on a 2.5-cm² screen contained in an open 9-cm-diameter petri plate. The fruits were gently misted with sterile distilled water delivered from an atomizer prior to placement in the mist chamber. Eight fruits were sampled for sporangial formation at 0, 3, 6, 12, 16, and 24 hr wetness durations at 10, 12.5, 15, 20, 25, 27.5, and 30 C. The order of temperatures tested was random. Temperature and wetness were continuously monitored within the mist chamber with thermistors and printed-circuit wetness sensors (Wong Labs, Cincinnati, OH 45200) connected to a second datalogger.

The 14-hr photoperiod was maintained throughout the experiment because, in nature, fruit are never in continuous darkness. The fruits were removed from the incubation jars at the conclusion of a 14-hr light period and placed in the mist chamber for a 10-hr dark period. Lights in the mist chamber were turned on after 10 hr. Light intensity within the mist chamber was 39 W/m².

Sporangial counts were made by removing eight fruits and their respective petri plates at random from the mist chamber at the above listed times. The mist that settled into each plate was added to a 50-ml graduated cylinder and brought to a final volume of 30-ml with sterile distilled water. Each fruit and respective 30-ml suspension were transferred to 50-ml plastic centrifuge tubes. Tubes were sealed with #6 rubber stoppers and vortexed at high speed for 2 min. Wash suspensions were transferred to 9-cm-diameter plastic petri plate bottoms and allowed to settle for 5 min. Sporangia at the bottom of plates were counted at ×40. Twenty fields were counted in each plate. Fruit length, diameter at calyx end, and weight were recorded for each fruit. The experiment was repeated.

Data analyses. Because strawberry fruit did not have identical sizes, sporangial production was divided by fruit surface area to calculate sporangia per square millimeter. As fruit shape was generally conical, the formula for surface area of a cone

$$S = \pi r s \qquad (1)$$

in which r = fruit radius (mm) and s = fruit slant height (mm), was used to determine fruit surface area.

Regression analysis was used to determine the effect of temperature (T) and wetness duration (W) on the number of sporangia (Y) produced on the surface of strawberry fruit infected with *P. cactorum*. Properties of the regression model had to include: an optimum relationship between Y and T, in which Y increases to a maximum and then declines; and a positive (monotonically increasing) relationship between Y and W. A model was chosen of the form

$$\ln(Y) = f(T, W) \qquad (2)$$

in which $\ln(Y)$ is the natural logarithm of Y and $f(T, W)$ represents an arbitrary function of T and W. The following linear terms were evaluated for making up $f(T, W)$: W, T, $W*T$, T^2, T^3, $W*T^2$, and $W*T^3$, in which "*" indicates multiplication. All possible combinations of these terms were evaluated for significance of the estimated parameters, coefficient of determination, and pattern of residuals (7–9). The regression analysis was performed on the data for each test separately, and then on the combined data. An F-test was conducted to determine if the results from the two tests were significantly different (9).

RESULTS

Sporangial production occurred between 12.5 and 27.5 C, with an optimum temperature of about 20 C (Fig. 1). In general, sporangial production increased with increased wetness duration between between 12.5 and 27.5 C (Fig. 2). Sporangia were absent at 10 and 30 C, although mycelial growth was profuse at 30 C. At 12.5 C, sporangia were first evident after 12 hr of wetness; 24 hr resulted in 49 sporangia per square millimeter (Fig. 2). Three hours of wetness resulted in less than 25 sporangia per square millimeter at 15, 20, 25, and 27.5 C, respectively. Sporangial production

increased to 523, 589, 150, and 49 sporangia per square millimeter at 15, 20, 25, and 27.5 C after 24 hr of wetness, respectively. One hundred or more sporangia were produced between 15 and 25 C with ≥16 hr of wetness. Only at 24 hr were more than 200 sporangia per square millimeter observed.

Data analyses. The best model representing the sporulation data was of the form

$$\ln(Y) = b_0 + b_1 T + b_2 W*T + b_3 T^3 + b_4 W*T^3 \qquad (3)$$

in which the bs are unknown parameters estimated from the data. Estimated parameters for the combined data, and for both tests, are presented in Table 1; all estimated parameters were significant ($P < 0.01$). An F-test indicated that there was no significant difference between the two tests ($P > 0.60$). The residuals had a random pattern and were normally distributed (9). There were no systematic deviations of observations from the model predictions for datum points corresponding to the dark or light regimes. The coefficient of determination was fairly high ($R^2 = 0.854$) for the combined data. The coefficient of determination adjusted for degrees of freedom (R_a^2), which can be considerably lower than R^2 if a model was to contain redundant or unnecessary terms, was almost as large (8–9). Both coefficients are reflective of the goodness of fit between observed and predicted log values. The coefficient of determination for the agreement between observed and actual sporangia values (R^{*2}) was determined to be 0.79 for the combined data.

The use of logarithms stabilized variances and linearized the relationship between sporangial numbers and wetness duration. T and T^3 terms in the model accounted for the observed optimum-type relationship between $\ln(Y)$ and temperature. There was also
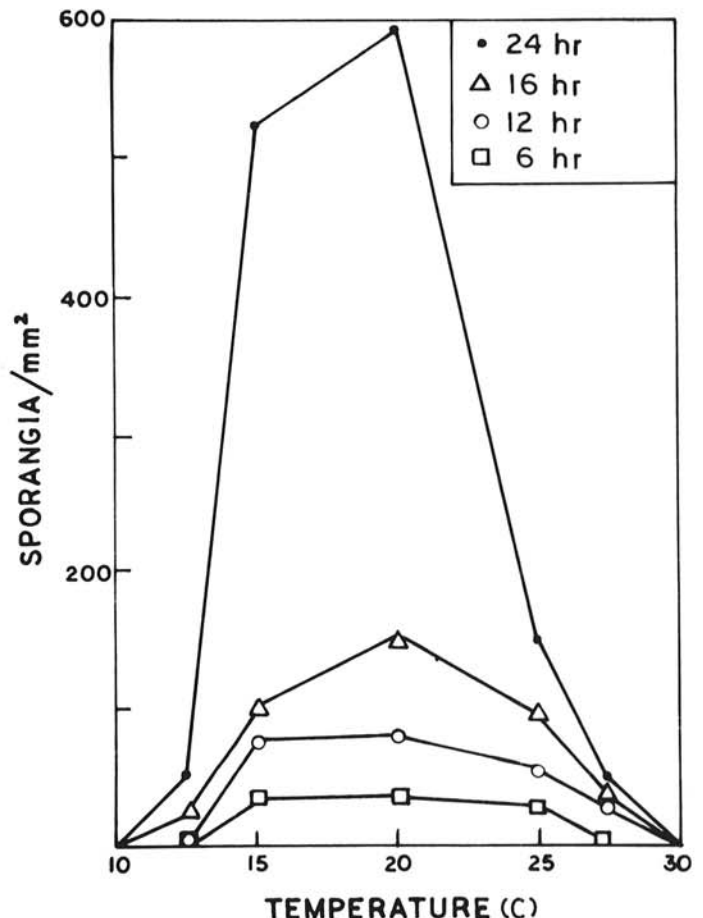

Fig. 1. Average sporangial production by *Phytophthora cactorum* on infected strawberry fruit surfaces over a range of temperatures and four wetness durations.

an interaction of W and T, as well as W and T^3. This indicated a differential response of $\ln(Y)$ to T, depending upon wetness duration.

Predicted values of $\ln(Y)$ were calculated for wetness durations between 0 and 24 hr and for temperatures between 12.5 and 27.5 C. Based on the terms in equation 3, bell-shaped curves were produced when $\ln(Y)$ was plotted vs. T, and straight lines were produced when $\ln(Y)$ was plotted versus W (Figs. 3 and 4). The interaction of W and T is clearly shown in Fig. 4 by the crossing prediction lines. The predicted T optimum can be calculated by taking the first derivative of equation 3. Although dependent on W, the optimum T varied by less than 1 C between 6 and 24 hr of wetness. The predicted optimum equaled 20.6 at 12 hr and 20.2 C at 24 hr.
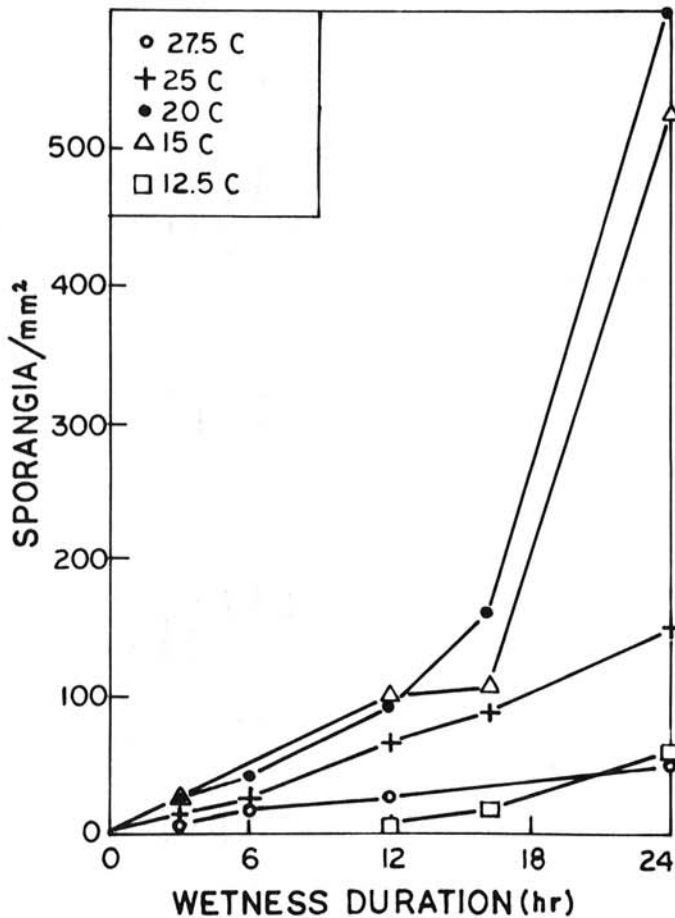
DISCUSSION

Results clearly indicated that wetness duration (W) and temperature (T) are significant environmental factors influencing sporulation of *P. cactorum* on infected strawberry fruit. Equation 3 was accepted as the best model for predicting the natural log of sporangia at values of W between 3 and 24 hr and T between between 12.5 and 27.5 C. Coefficients of determination of 0.87, 0.84, and 0.85 for tests one and two and the combined data, respectively, indicate that a relatively high proportion of the variability in sporangial numbers in the tests was accounted for by the components of the model. The similarity of R^2 and R_a^2 values is further evidence of the importance of the various components in the model (7–9). An interaction between W and T was also evident upon inspection of the model. As wetness duration increased, the range of temperatures over which high sporulation occurs broadened. We would not expect $\ln(Y)$ to increase in a linear fashion with W much beyond 24 hr. Obviously, the numbers will level off as maximum sporangial production is reached. However, wetness durations of less than 24 hr are more typical of field conditions, and the log transformation was appropriate. Other models, such as a generalization of Schrodter's sine model (7), were tested for fitting to the sporangia data. None of these fit the data as well as equation 3.
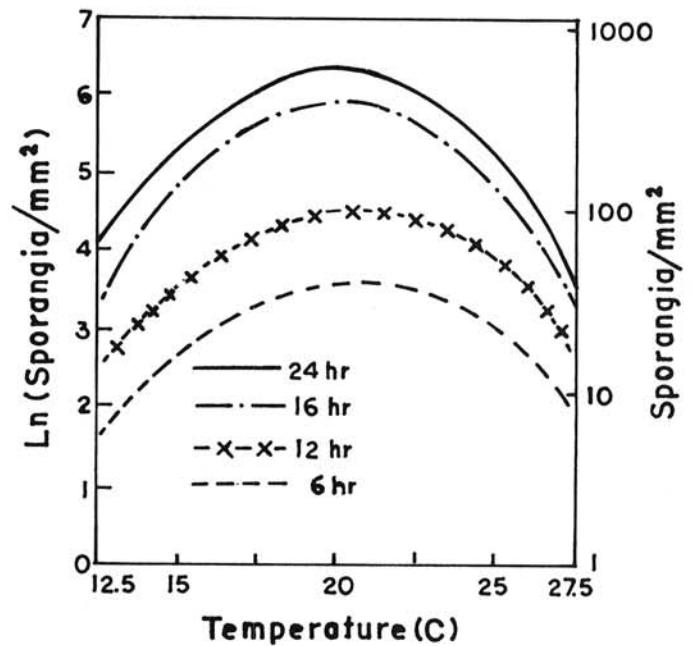

Fig. 2. Average sporangial production by *Phytophthora cactorum* on infected strawberry fruit surfaces over a range of wetness durations and five temperatures.

Fig. 3. Effect of temperature on predicted numbers of sporangia produced by *Phytophthora cactorum* on infected strawberry fruit at four wetness durations. The curves were generated by using equation 3 with parameters listed in Table 1 for the combined tests.

TABLE 1. Estimated parameters of equation 3 for temperature (T) and wetness duration (W) effects on sporangial production of *Phytophthora cactorum*, together with the coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R_a^2), and the standard error about the regression curves (S)

	Estimated parameters[y]					R^2	R_a^2	S
	b_0	b_1	b_2	b_3	b_4			
Test 1	-6.11^z (1.54)	0.621 (0.120)	1.3×10^{-2} (2.2×10^{-3})	-4.6×10^{-4} (9.6×10^{-5})	-1.3×10^{-5} (3.8×10^{-6})	0.870	0.839	0.54
Test 2	-5.17 (1.67)	0.554 (0.130)	1.3×10^{-2} (2.5×10^{-3})	-4.0×10^{-4} (1.1×10^{-4})	-1.3×10^{-5} (4.3×10^{-6})	0.837	0.796	0.58
Combined tests	-5.68 (1.06)	0.589 (0.082)	1.3×10^{-2} (1.6×10^{-3})	-4.3×10^{-4} (6.8×10^{-5})	-1.3×10^{-5} (2.6×10^{-6})	0.854	0.838	0.53

[y] Estimated parameters for equation 3 corresponding to intercepts (b_0), T (b_1), $W*T$ (b_2), T^3 (b_3), and $W*T^3$ (b_4). Numbers in parentheses under the parameters correspond to their standard deviations. Temperature is in centigrade, wetness is in hours.
[z] b_0 is the value of $\ln(Y)$ when $T = 0$ and $W = 0$; value of Y at these conditions is equal to $\exp(b_0)$.
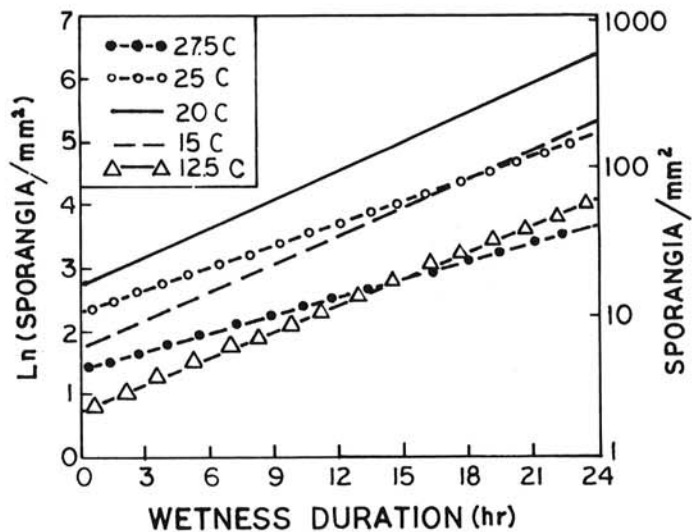
Fig. 4. Effect of wetness duration on predicted numbers of sporangia produced by *Phytophthora cactorum* at five temperatures. Curves were generated by using equation 3 with parameters listed in Table 1 for the combined tests.

Results obtained in this study differ somewhat from findings of Rose (12) who reported a temperature optimum for sporulation in vivo falling between 10 and 20 C. Since we observed no sporulation at 10 C, our temperature findings appear somewhat higher. However, the optimum sporulation temperature and the temperature range over which sporulation occurs could be influenced by pathogen isolate, host cultivar, or other environmental conditions (14).

Although direct comparison between in vitro and in vivo sporulation parameters are at best artificial (14), free water has been reported to enhance sporulation of *P. cactorum* grown on a solid medium; exposure of 24-hr-old cultures to 12 hr of free water resulted in significant increases in sporangial number (18). When using the closed-system for controlling RH, we were unable to find more than an occasional sporangium per fruit, even at 100% RH (*unpublished*). Those sporangia were probably formed where water condensed on the fruit. Our results indicated that a film of free water was required for in vivo sporangial production and that the sporulation response was first detected after a wetness duration of 3 hr between 15 and 25 C.

Temperature and moisture requirements for sporangial production in vivo are quite variable among other *Phytophthora* spp. Trujillo (17) reported that sporangial production of *P. colcasiae* occurred after 2–3 hr at 100% RH. *P. infestans* has been reported to sporulate between 3 and 26 C (1). Eight-hour periods of 100% RH within the optimal temperature range of 18–22 C were required for sporulation on infected potato (*Solanum tuberosum*) tissue. Sporulation at temperatures outside this range required progressively longer exposure to 100% RH. Rocha and Maihado (10) reported sporangial formation in *P. palmivora* occurred at 70–90% RH, but not at 100%. Gerlach et al (3) reported sporulation on *Pieris japonica* infected with *P. citrophthora* after 4 hr of immersion in salt solutions at 16–28 C. However, a minimum of 7 hr of immersion was needed at 8 and 32 C. Temperature and moisture requirements and their interactions are evidently somewhat unique to each pathosystem.

The influence of temperature and wetness duration on infection of strawberry fruit by *P. cactorum* has been reported (5). A regression model with identical *W* and *T* terms as for sporangia production (eq. 3) was developed for describing infection as a function of *T* and *W* (equation 7 of reference 5). Wetness periods required for infection were exceedingly short, eg, >80% of the fruit were infected between 17 and 25 C with a wetness duration of 2 hr. Due to the shortness of the wetness periods required for infection and the relatively frequent occurrence of these conditions during the Ohio strawberry season, we felt that other disease cycle components were the determining factors for leather rot epidemics. Although sporangia form after a relatively brief wetness period (3 hr) at 15–25 C, sporangia are much more abundant at longer wetness durations. We feel that sporangia production and subsequent splash dispersal (4) are key components for the development of epidemics. We plan to incorporate our results into a predictive system for scheduling fungicide applications.

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